Preclinical Lupus

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Patients who have autoantibodies associated with SLE (e.g. positive ANA, anti-dsDNA) but no symptoms can be classified as preclinical SLE

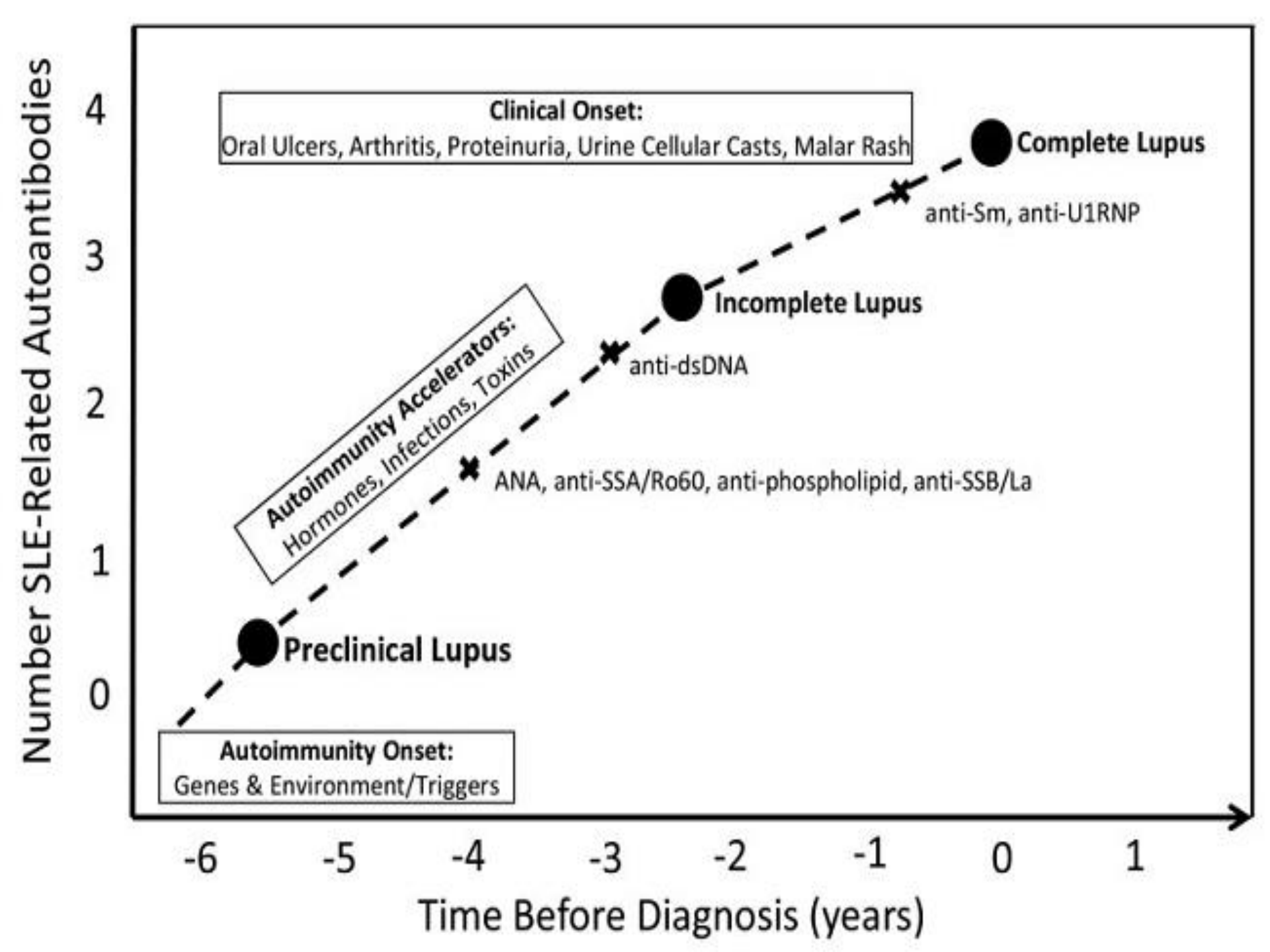
How much autoantibodies important?

- Autoantibodies can be present up to 10 years before the onset of clinical symptoms.
- This phenomenon is not limited to SLE, but has also been shown in studies of preclinical rheumatoid arthritis (RA),as well as in type 1 diabetes mellitus
- Just detecting these auto antibodies will not be sufficient to predict disease, because ANA positivity, at least at low levels, is greater than 25%in normal people, whereas SLE has a prevalence that is probably less than 0.15%

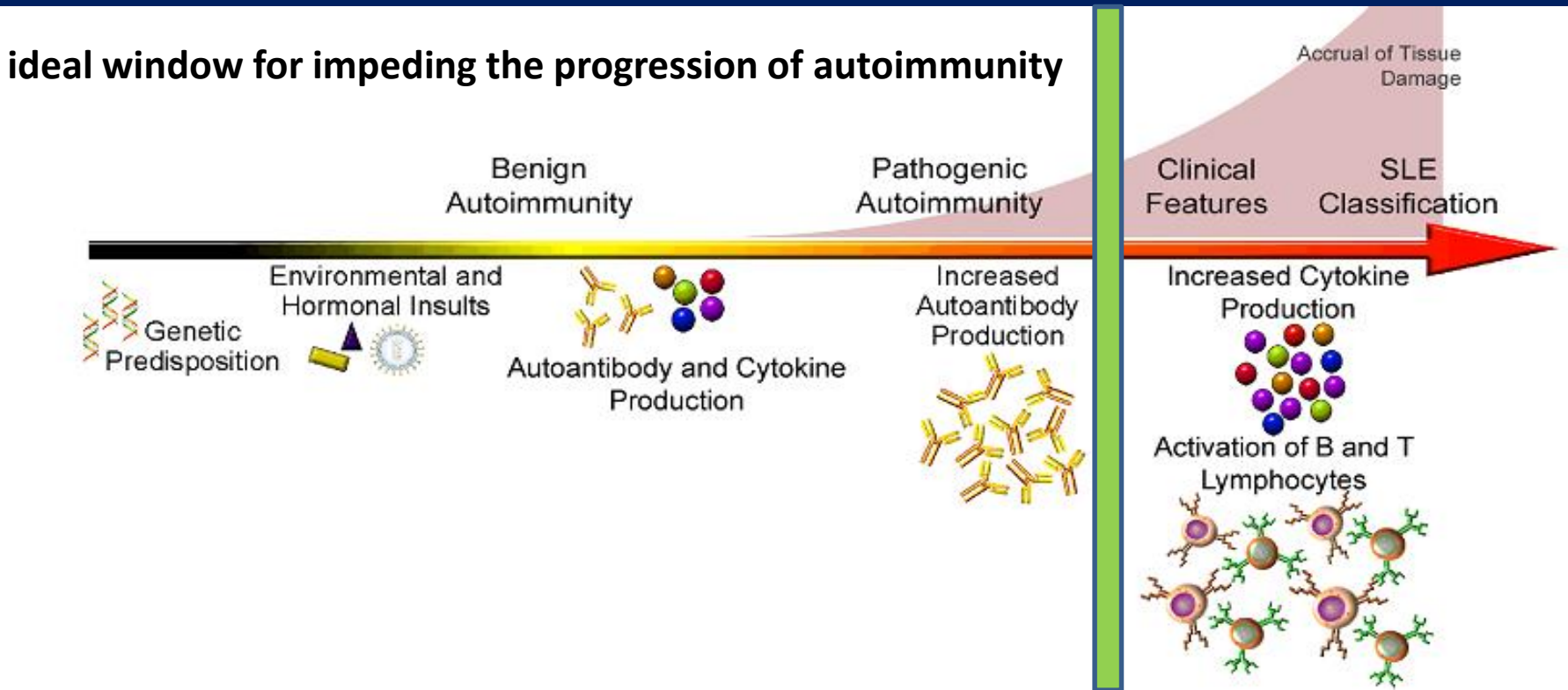
partnership with US military rheumatologists and the US Department of Defense Serum Repository (DoDSR), a large sample repository comprised of longitudinal blood samples and basic laboratory evaluations were obtained on entry into the military and throughout military service

- 130 subjects who subsequently developed SLE while in the US military, 115 (88%) of the SLE subjects were found to have at least one autoantibody present in a prediagnosis serum sample. In some cases, this initial autoantibody was present up to 9.4 years
- Anti-phospholipid, anti-Ro, anti-La, and anti-nuclear antibodies (ANAs) were present significantly earlier (mean 5 3.2 years) than anti-Sm and anti–nuclear ribonucleoprotein (anti-nRNP) antibodies (1.2 years) (P 5 .005). Anti–double stranded DNA (anti-dsDNA) antibodies appeared, on average, 2.2 years before diagnosis, whereas anti-ribosomal P and anti-C1q antibodies were detectable, on average, 1.1 and 1.4 years before classification, respectively

Heinlen LD J Ribosomal P autoantibodies are present before SLE onset and are directed against non-C-terminal peptidesMol Med (Berl). 2010 Jul;88(7):719-27.

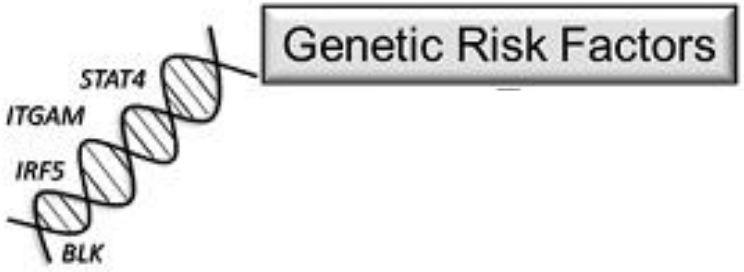

Number SLE-Related Autoantibodies
4
3
2
1
0
Clinical Onset:
Oral Ulcers, Arthritis, Proteinuria, Urine Cellular Casts, Malar Rash
Complete Lupus
anti-Sm, anti-U1RNP
Incomplete Lupus
anti-dsDNA
Autoimmunity Accelerators:
Hormones, Infections, Toxins
ANA, anti-SSA/Ro60, anti-phospholipid, anti-SSB/La
Preclinical Lupus
Autoimmunity Onset:
Genes & Environment/Triggers
-6
-5
-4
-3
-2
-1
0
1
Time Before Diagnosis (years)

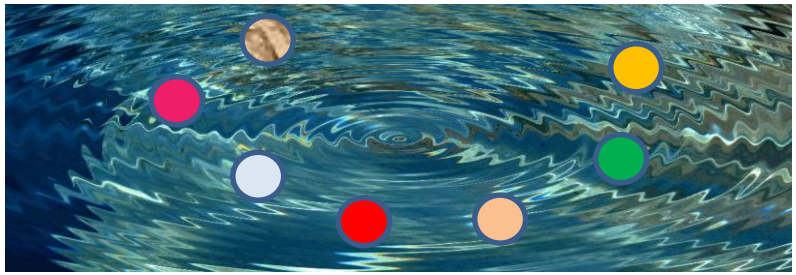
Autoimmunity does not occur overnight



Pathophysiology important

Because reversing autoimmunity in early stage may prevent lupus presentation

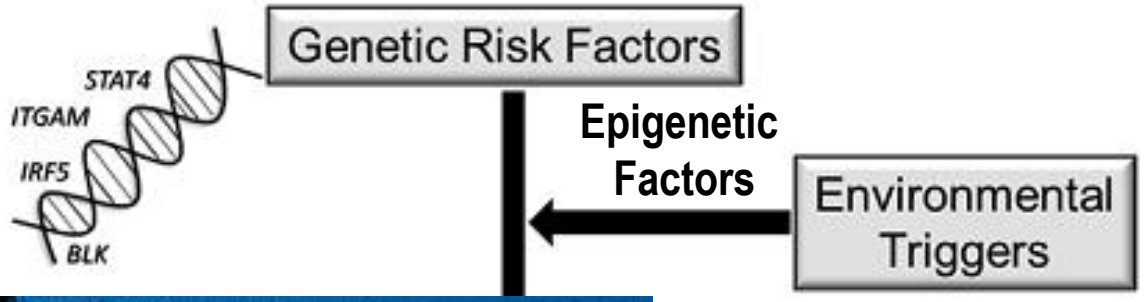




MHC class III
MHC class II
IRF5
ITGAM
STAT4
PTPN22
TNFAIP3
TNFSF4
FCGR3B
BLK
FCGRA
TNIP1
IRAK1/MECP2
ATG5

Pathophysiology important

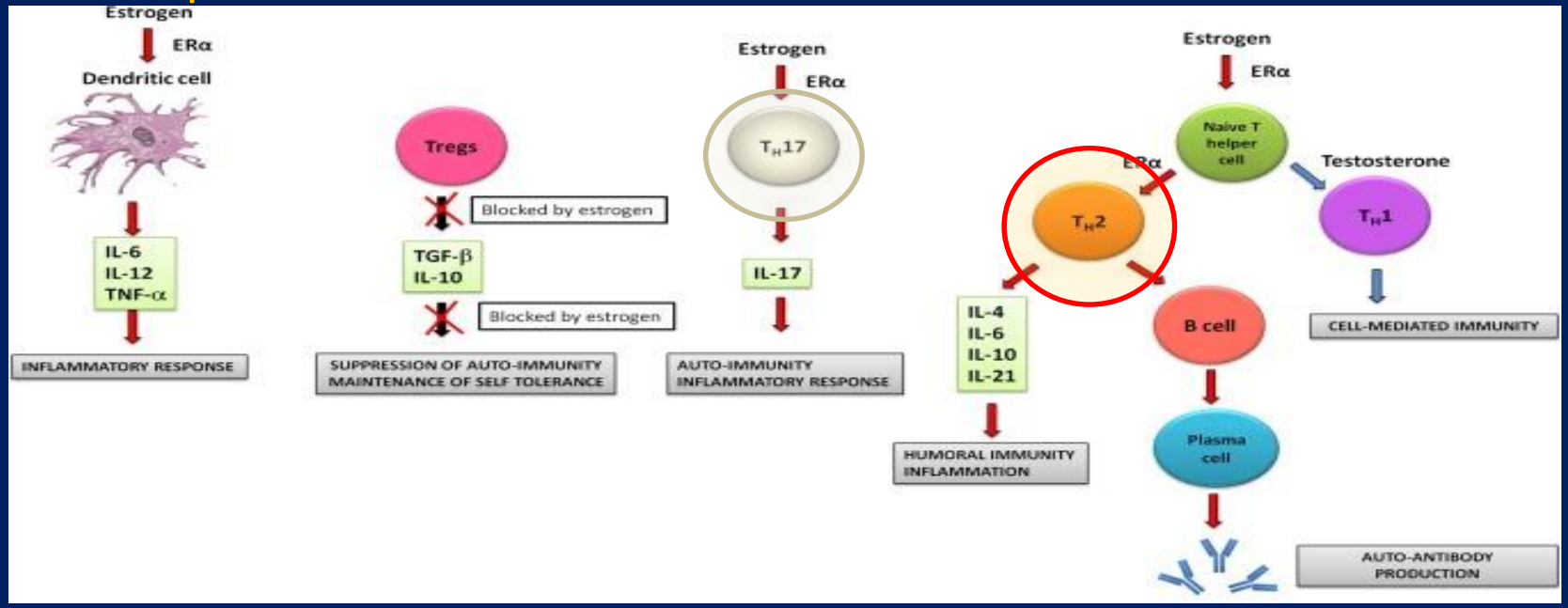
- The inheritance of genes alone is not sufficient for developing SLE, suggesting the influence of environmental triggers on disease
- Although first degree relatives of patients with SLE overall have a higher prevalence of autoantibodies and a higher risk of SLE and other autoimmune diseases, some develop SLE-specific autoantibodies but never develop clinical disease



- Hormones
- Microbes
- Ultraviolet light
- Diet
- Toxins
- Stochastic effects

Estrogens

- Estrogens are immunostimulatory in that they induce polyclonal expansion and proliferation of B-cells in physiological doses
- Recent data suggest that the role of estrogens in the pathogenesis in lupus is potentially complex and age dependent
- At a supraphysiological dose, estrogens are able to inhibit IL-2 production. Thus, they theoretically induce the higher susceptibility of SLE in females of reproductive age or supplement, because of inhibition of Th1 responses, CD40L upregulation and bias towards Th2 responses



Smoking

- It should be appreciated that the detrimental effects of cigarette smoking also apply to marijuana smoking.
- Smoking increases disease activity and is reported to interfere with the action of antimalarial drugs.
- Its main effects occur in the context of HLA-DR3 alleles.
- Genetically predisposed subjects with a smoking related decreased ability to clear apoptotic cell
- Smoking is related to chronic inflammation, documented by an increase in inflammatory markers such as C-reactive protein, adhesion molecules and selectines, production of pro-inflammatory cytokines, and stimulation of autoreactive B- and T-lymphocytes
- Meta-analysis of Costenbader *et al* showing that **current smoking** rather than former smoking is most important in risk assessment for SLE development.

Drugs

- More than 80 drugs have been associated with this adverse event, of which some (i.e., procainamide and hydralazine) confer a high risk while others (i.e., quinidine, anti-TNF) are associated with a relatively lower one
- Biologic-related DISLE (TNFB,IFN)may manifest with renal and dermatologic symptoms, hypocomplementemia, high titers of anti-dsDNA antibodies, and low titers of anti-histone antibodies

Vaccines

- similarly to drugs, vaccines are rarely linked to autoimmune phenomena via mechanisms that depend on different ingredients of the vaccine
- Other components such as adjuvants (e.g., aluminum) may activate both the innate and adaptive immune responses, as was outlined in animal models and human studies of the ASIA syndrome
- Association between SLE and immunization, particularly with hepatitis B and human papilloma vaccines, has been reported

Infections

- Infections have been implicated in SLE for many years
- EBV and CMV , parvovirus B19, HRES-1 are considered to be SLE triggers
- whereas Helicobacter pylori, hepatitis B virus, and parasite infections are thought to be protective

Mechanisms of protection infection

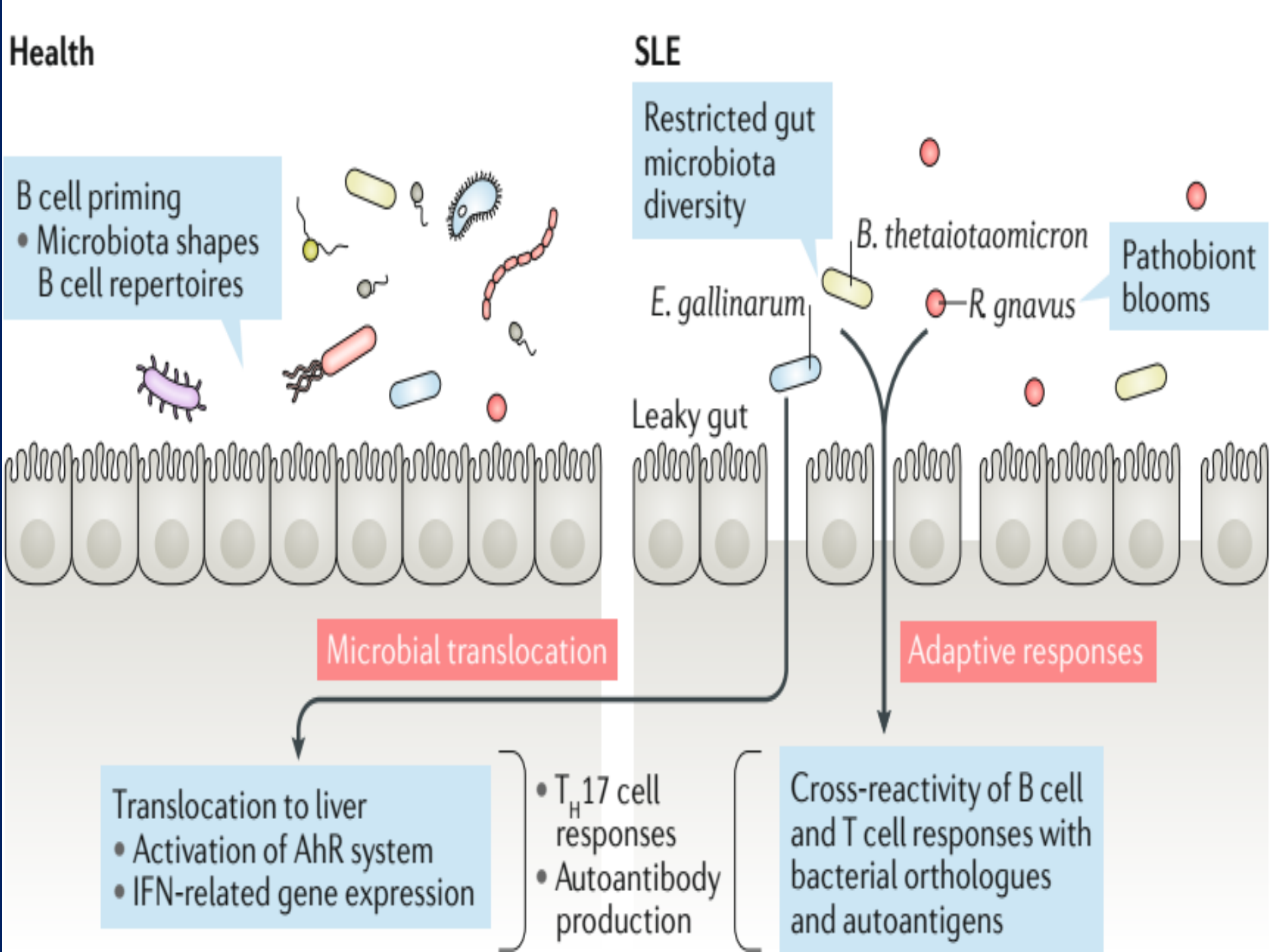
Improvement in hygiene and absence of certain microbes may have contributed to the higher incidence and faster progression of lupus disease

Qinghui MuSLE: Another Autoimmune Disorder Influenced by Microbes and Diet? Front Immunol. 2015; 6: 608.Published online 2015 Nov 30.

Microbion

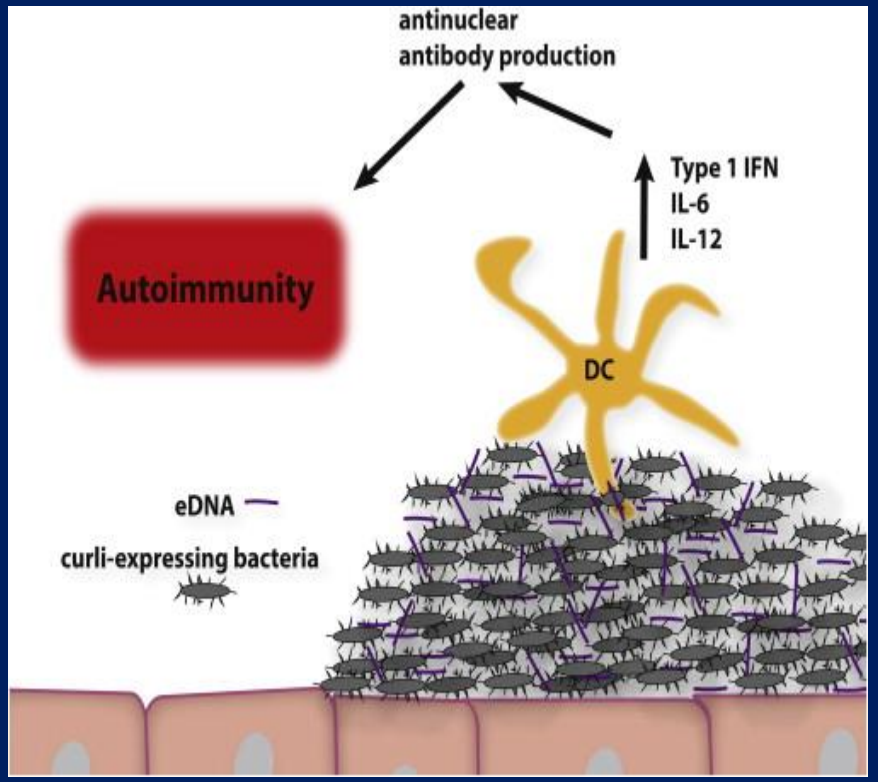
- Manfredo et al reported that translocation of a commensal bacterium, *Enterococcus gallinarum*, from the small intestine to the liver causes a lupus-like condition in autoimmunity-prone mice by induce T helper 17 cell generation, resulting in a systemic type I interferon signature and anti-dsDNA antibody production

Manfredo Vieira, S. et al. Translocation of a gutpathobiont drives autoimmunity in mice and humans.*Science* **359**, 1156–1161 (2018).


Health
SLE
B cell priming
• Microbiota shapes B cell repertoires
Restricted gut microbiota diversity
B. thetaiotaomicron
E. gallinarum
R gnavus
Pathobiont blooms
Leaky gut
Microbial translocation
Adaptive responses
Translocation to liver
• Activation of AhR system
• IFN-related gene expression
• T_H17 cell responses
• Autoantibody production
Cross-reactivity of B cell and T cell responses with bacterial orthologues and autoantigens

Infections

- Bacterial biofilms represent another mechanism by which microorganisms interact with the immune system.
- Amyloid–DNA (curli)complexes, found in many biofilms, producing high levels of inflammatory molecules such as type 1 interferons that greatly increased the production of autoantibodies in lupus prone mice
- At this point, the evidence seems clear that SLE is not uniformly caused by a single infection, but the role of bacteria and viruses generally in SLE represents an emerging area of study, and TLR antagonists are being evaluated as therapeutic agents



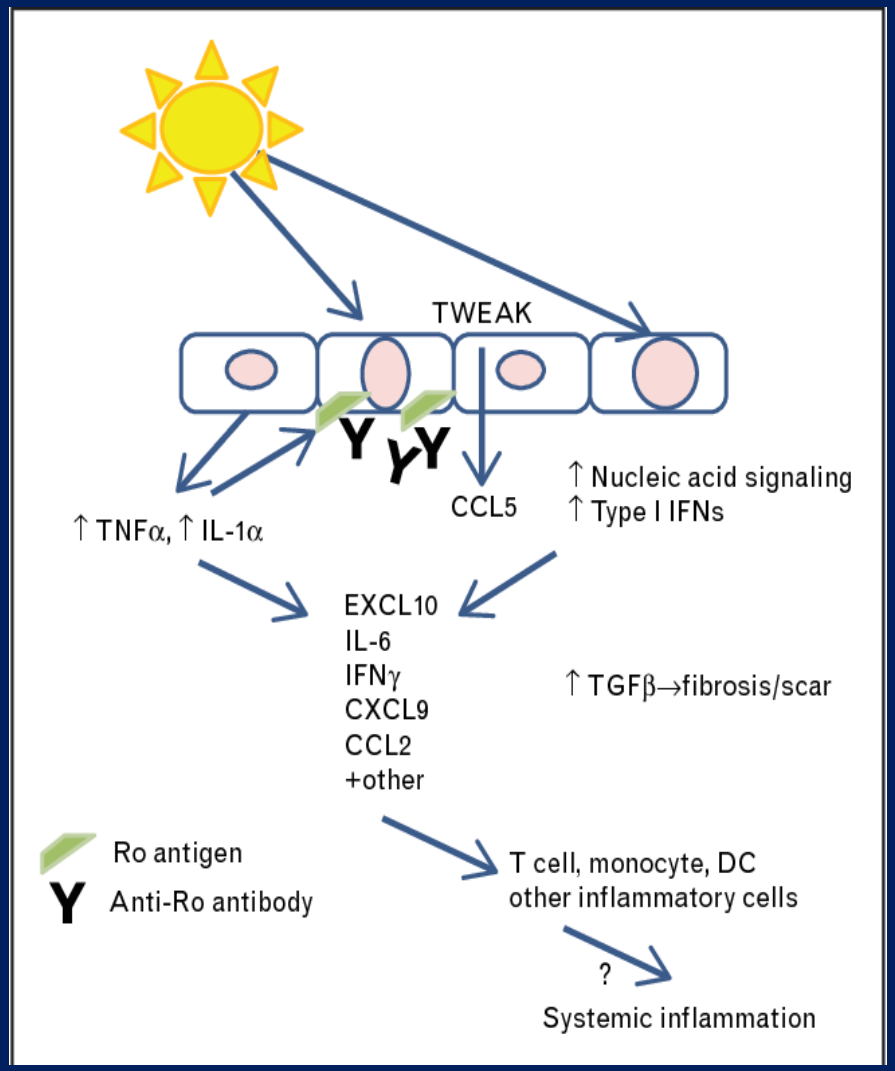
Gallo PM Immunity. 2015 Jun 16;42(6):1171-84. doi: 10.1016/j.immuni.2015.06.002.
Amyloid-DNA Composites of Bacterial Biofilms Stimulate Autoimmunity.

Antibiotics and SLE

- Antibiotics, which can remove gut bacteria, are known to trigger lupus flares. These include sulfa drugs such as trimethoprim–sulfamethoxazole (Septra), tetracycline-related antibiotics such as minocycline, and penicillin-related antibiotics such as amoxicillin.
- However, antibiotics also cause diarrhea and remove beneficial microbes from the intestinal tract
- Increased sun sensitivity with antibiotics may be one mechanism behind the observations.

Ultraviolent Light

- It is evident that UV-B induces apoptosis of keratinocytes and other cells
- Releases a large amount of autoantigens and pro-inflammatory cytokines to the circulation, triggering autoimmune-related systemic inflammation



Immunometabolism

Is a relatively new field Insufficient energy or metabolite levels, can trigger cell death. This can lead not only to cellular imbalance, such as lymphopenia in lupus, but also to a rich source of autoantigens in the form of cellular debris provides substrates for epigenetic modifications of DNA and histones

Yaima L. Metabolic Abnormalities and Oxidative Stress in LupusCurr Opin Rheumatol. 2017 Sep; 29(5): 442–449.

Immunometabolism

- Pathogenic T cells take on a chronically activated phenotype, and thus have dramatically different metabolic needs than healthy tissue
- CD4 T cells in SLE meet their energetic needs mostly through oxidative phosphorylation
- Oxidative stress, also contribute to accelerated atherosclerosis and cardiovascular events in this disease
- This augmented reliance on oxidative phosphorylation is coupled to increased mitochondrial polarization and mass in the T cells of SLE patients, which is caused in part by decreased mitochondrial autophagy
- Metabolic therapy is able to prevent disease, because there is a substantially decreased production of autoantibody and formation of pathogenic central memory T cells in the treated mice

Ian A. Targeting Metabolism as a Novel Therapeutic Approach to Autoimmunity, Inflammation, and TransplantationJ Immunol February 1, 2017, 198 (3) 999-1005

Diet

- Epigenetic changes in response to diet and other environmental exposures have important implications for the development of SLE, including potential targets for prevention
- Diet is known to influence DNA methylation, which may be one mechanism by which diet contributes to SLE susceptibility

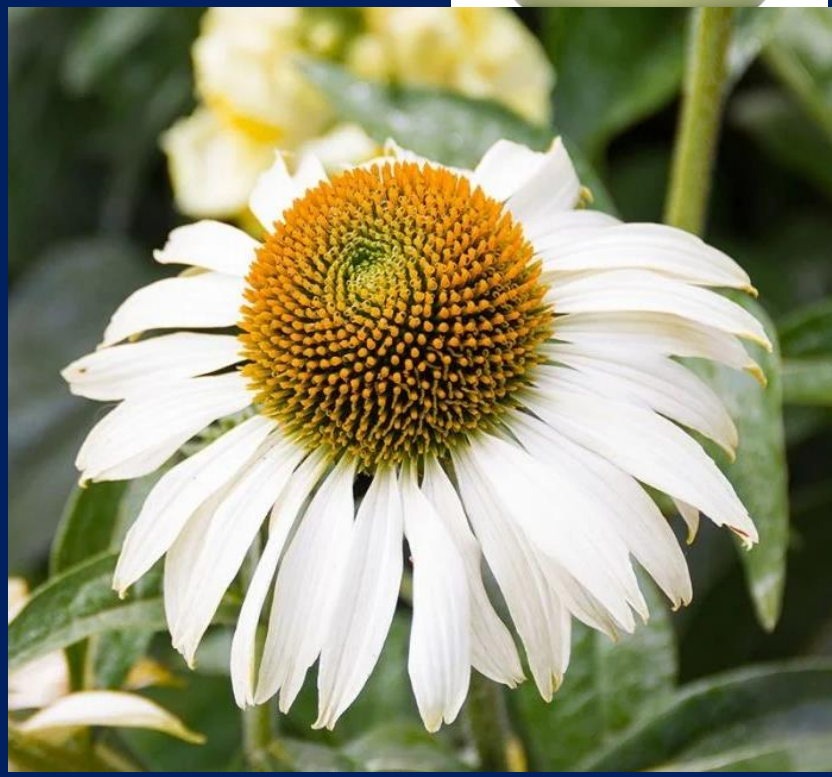
Alfalfa Seeds

بذر یونجه

- Alfalfa is primarily used in the United States,Australia and New Zealand for dairy production, beef and lamb.
- However, it is also used for human consumption, particularly as a salad ingredient in these countries. Over the past few decades there has been interest and research into alfalfa's cholesterol lowering activities
- Further research examining the link between alfalfa and lupus concluded that the amino acid L-canavanine was the key constituent of alfalfa which exacerbated lupus, though a lack of control over autoantibody synthesis and lymphocyte proliferation.
- L-canavanine is found in many legumes including soyabean, alfalfa, clover and onions

Echinacea سرخارگل

- From the cone flower (Echinacea purpurea)
- Used to treat colds and flu
- Increases immune system activity Can worsen lupus
- Series of lupus patients at Johns Hopkins Hospital got worse while taking Echinacea and 2 patients required strong chemotherapy for lupus nephritis



Garlic

- Three substances in garlic allicin, ajoene, and thiosulfinates booster immune system by enhancing the activity of white blood cells, particularly macrophages and lymphocytes
- Many site prohibit garlic for lupus patients but there is no RCT

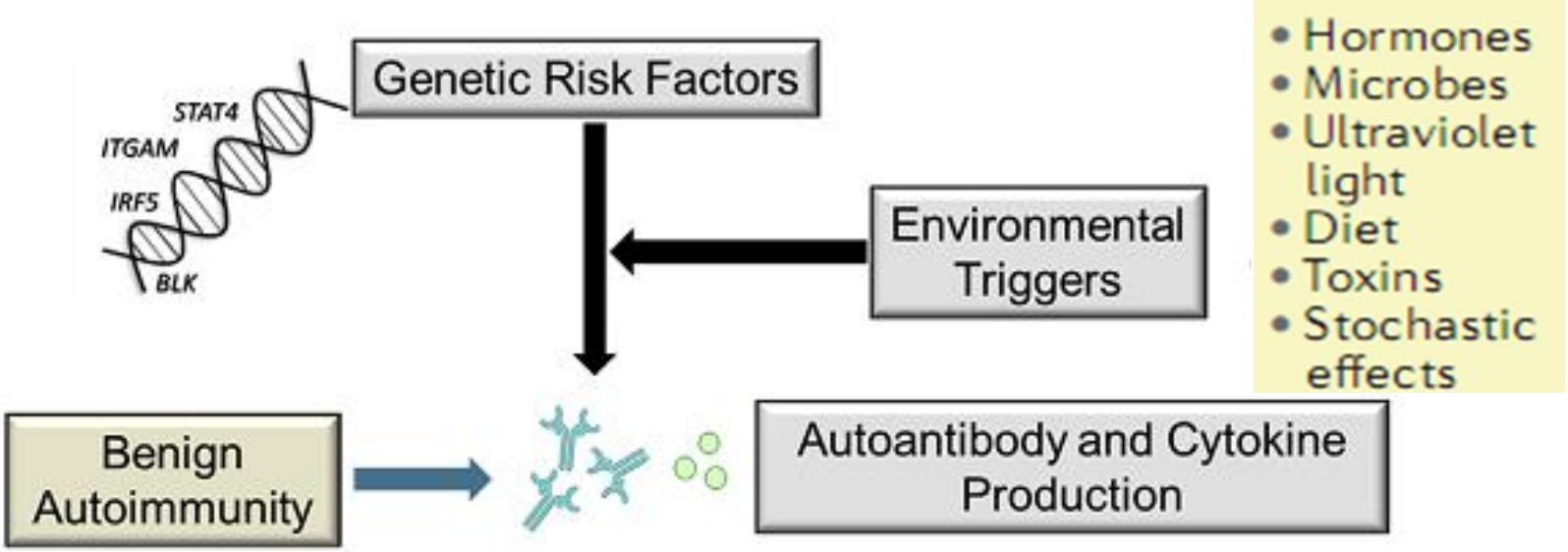
Omega 6

- The benefits of omega-3 polyunsaturated fatty acids in lupus have previously been outlined.
- Saturated or omega-6 polyunsaturated fats have been shown to have a detrimental effect on autoimmune disease activity and reduce survival in a number of animal models. Autoimmune-prone mice fed saturated fats appear to experience more severe nephritis and glomerular pathology, leading to the hypothesis that dietary fat, especially saturated fat, restriction may be an effective therapeutic approach to lupus nephritis.
- Research in humans is sparse, but one study followed patients with lupus who reduced their intake of omega- 6 polyunsaturated fats for one year. In this study, the number of patients with active disease fell from 11 to 3 but the study group was small
- Foods high in omega-6 fatty acids include oils such as sunflower oil, poppy seed oil, corn oil and foods such as mayonnaise, margarine and brazil nuts.

Vitamin D Deficiency

- Using chromatin immunoprecipitation followed by massively parallel DNA sequencing (ChIP-seq), 2776 binding sites for vitamin D receptor binding were found along the length of the human genome.
- Significantly, there was significant enrichment of these binding sites in regions associated with, including DNAase I-hypersensitive sites and specific histone modifications
- These results strongly imply that vitamin D, upon binding to its cognate nuclear receptor, exhibits a potential regulatory role in gene expression important for SLE pathogenesis and modulation of disease activity
- The importance of vitamin D in regulating innate and adaptive immunity has been highlighted by studies demonstrating inhibition of interferon α (IFN α)-mediated monocyte differentiation into dendritic cells, increased serum IFN α activity in SLE patients with vitamin D deficiency
- But data very conflicting

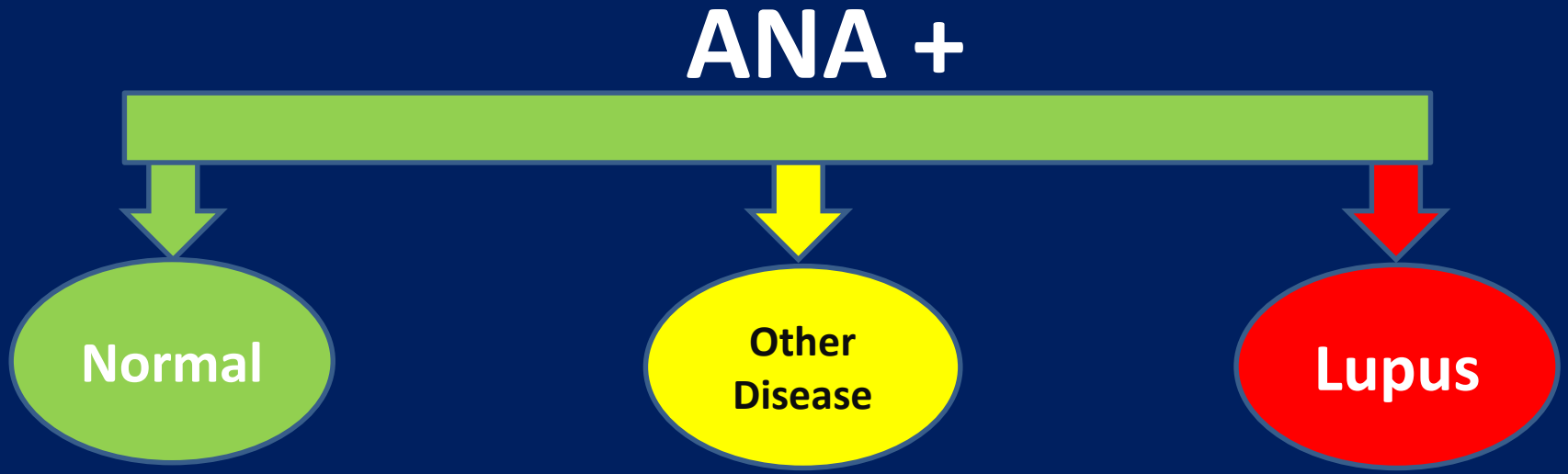
Cynthia AranowDouble-Blind Randomized Placebo-Controlled Trial of the Effect of Vitamin D3 on the Interferon Signature in Patients with Systemic Lupus ErythematosusArthritis Rheumatol. 2015 Jul; 67(7): 1848–1857.doi: 10.1002/art.39108



- Autoantibodies can be present up to 10 years before the onset of symptoms
- Detecting these autoantibodies will not be sufficient to predict disease, because the prevalence of ANA positivity in the general population exceeds the prevalence of SLE
- Prevalence of ANA positivity, at least at low levels,is greater than 25%,whereas SLE has a prevalence that is probably less than 0.15%

Autoantibody positivity

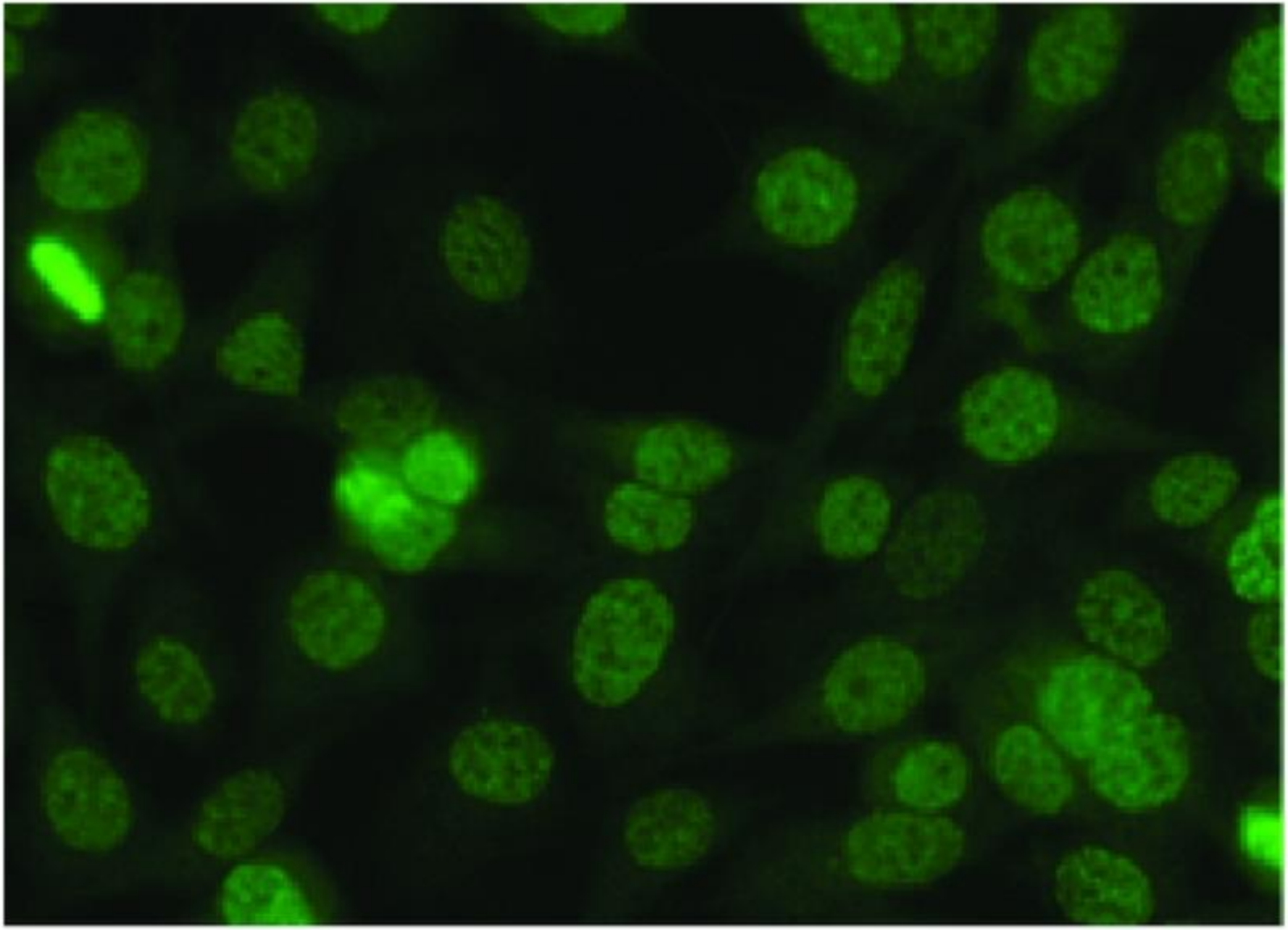
Therefore, it is of great interest and importance to understand what factors provide a positive ANA with predictive value for lupus



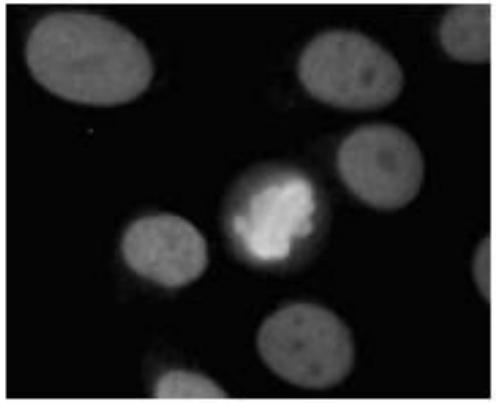
Autoantibodies without SLE Associations in healthy individuals

- Up to 25% of serum samples from healthy individuals have been reported to have a positive ANA test, the majority of which are directed to the dense fine speckles 70 (DFS70) antigen
- Serological profile of anti-DFS70 positive females required further clinical and laboratory investigations in order to define the presence of associated disease-marker autoantibodies and their clinical significance.
- In contrast, isolated anti-DFS70 specificity in male population suggests that the DFS70 could be considered reliable screening indicator for absence of other circulating autoantibodies resulting in considerable cost-saving potential

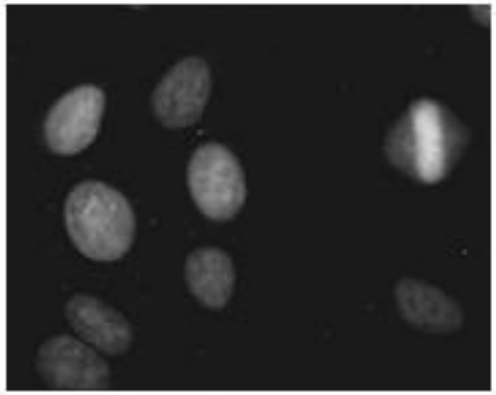
Prevalence and serological profile of anti-DFS70 positive subjects from a routine ANA cohortTeresa Carbone etal *Scientific Reports***volume 9**, Article number: 2177 2019



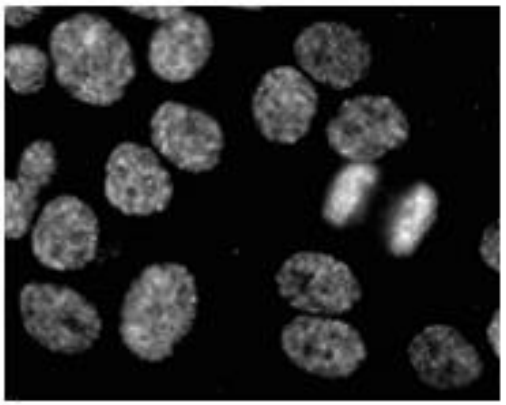
DFS sample 3



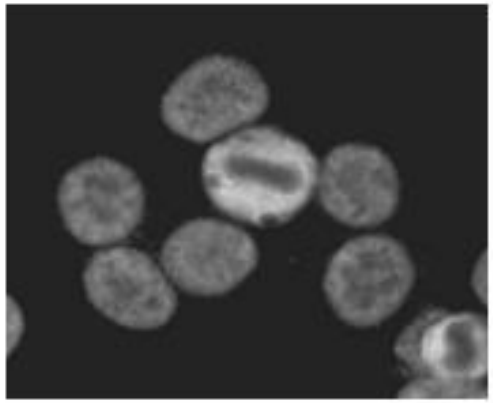
Homogeneous



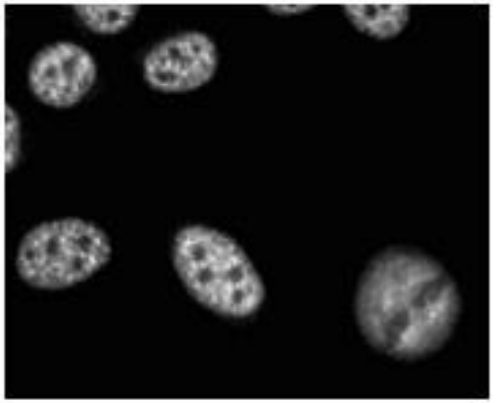
Quasi-homogeneous



Dense fine speckled

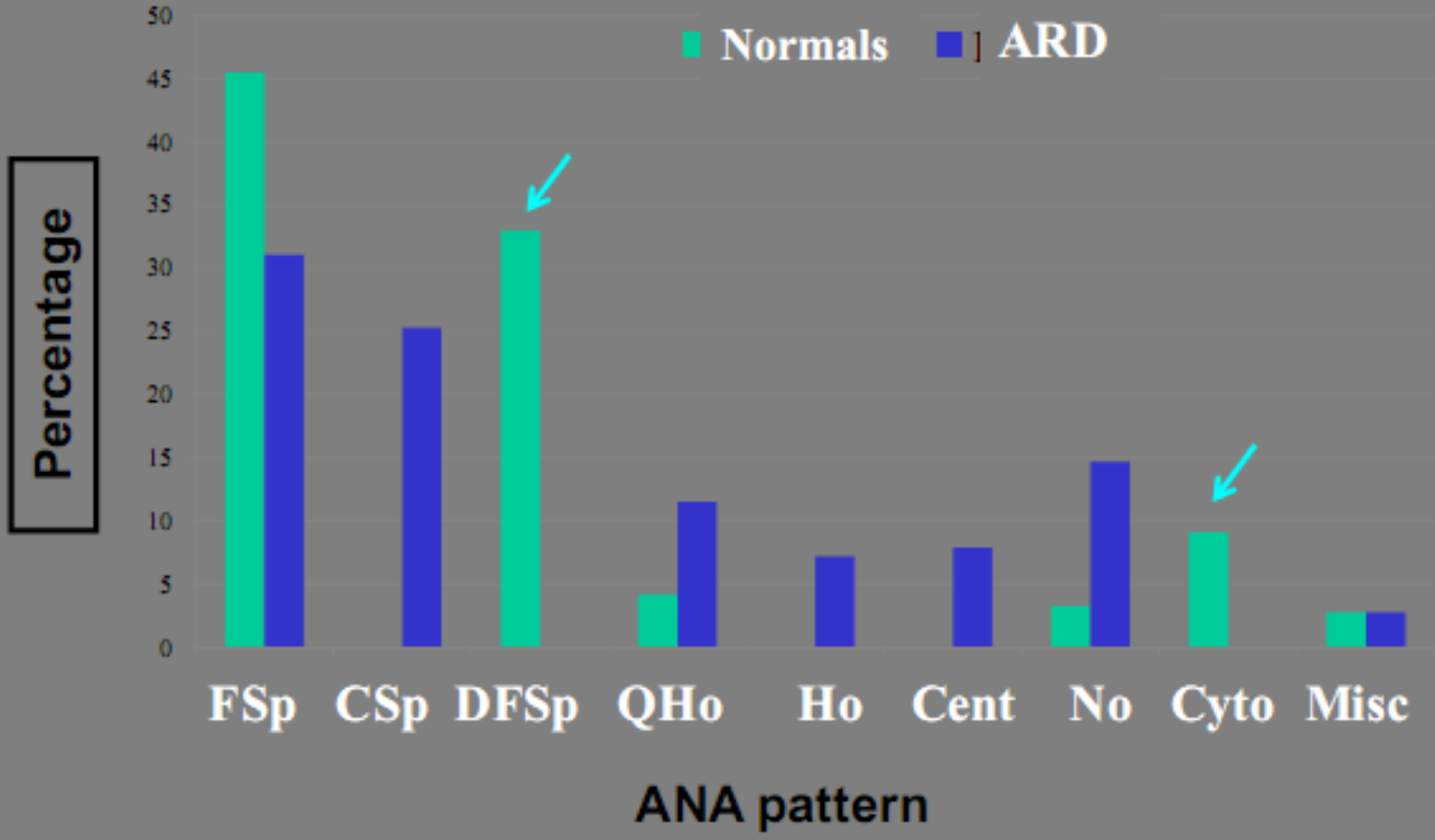


Fine speckled



Coarse speckled

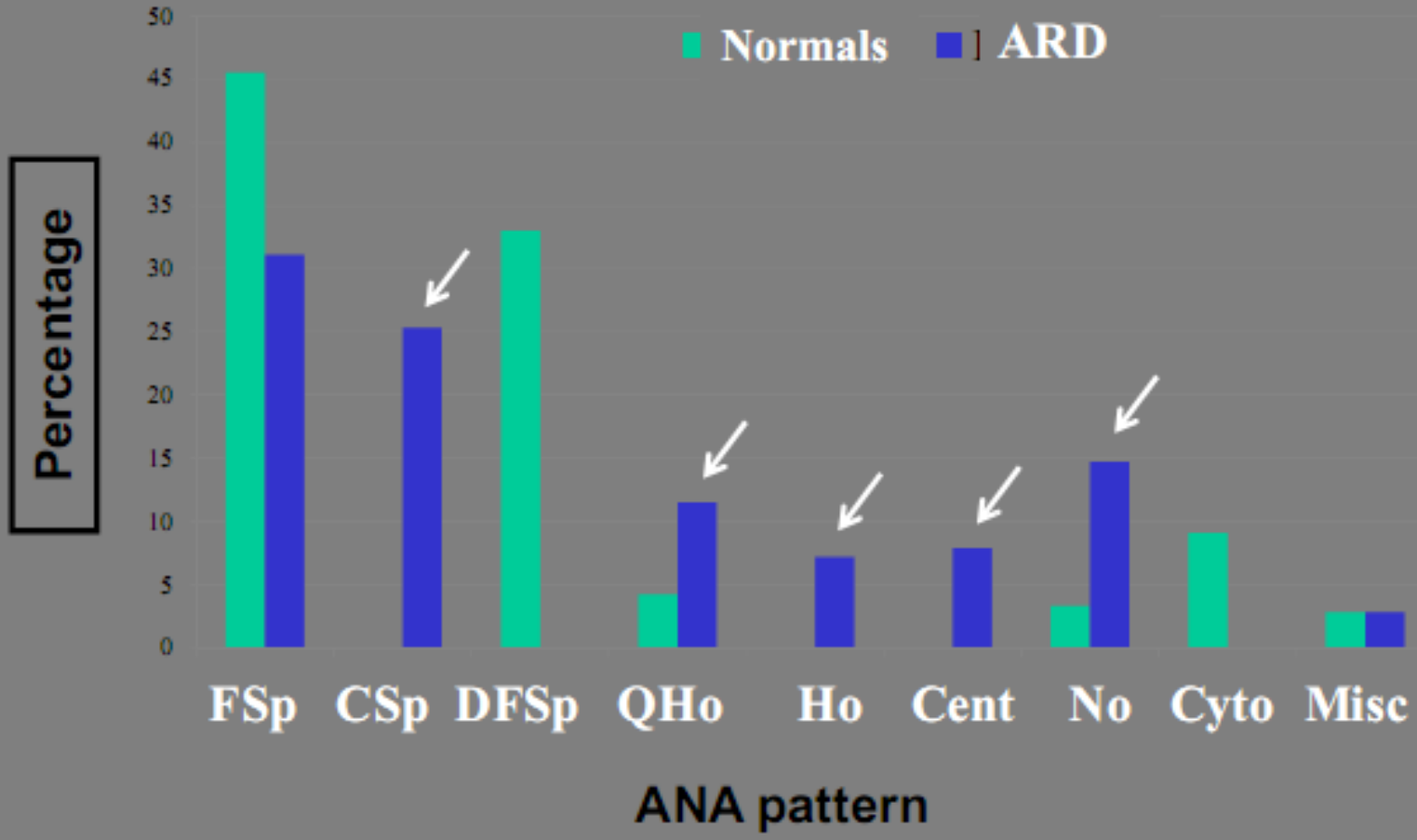
ANA-HEp-2 pattern in 918 healthy subjects and 153 patients with autoimmune rheumatic diseases



Healthy subjects → 12.8% positive ANA x ARD → 90.1% Positive ANA

Mariz HA et al, submitted

ANA-HEp-2 pattern in 918 healthy subjects and 153 patients with autoimmune rheumatic diseases



Healthy subjects ➔ 12.8% positive ANA x ARD ➔ 90.1% Positive ANA

Mariz HA et al, submitted

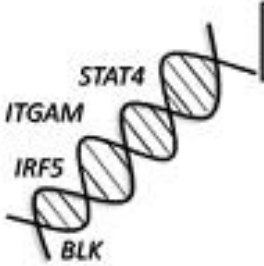
Autoantibodies without SLE Associations in other indolent disease

- A substantial number of individuals who are referred for rheumatology evaluation on the basis of ANA positivity might, in fact, have underlying thyroid autoimmunity.(ANA positive in 45-74%)
- Thyroid autoimmunity 10 times more common than SLE

Journal of immunology and research vol 2014 article 150239

Four-cytokine panel including BLyS, IL-2,IL-5, and G-CSF that could distinguish ANA+ healthy individuals from ANA- healthy individuals or lupus patients, thereby suggesting a method by which ANA+ individuals at higher risk of evolving to clinical disease could be identified, based on their deviation from this profile

Lu R, Munroe ME, Guthridge JM et al. Dysregulation ofinnate and adaptive serum mediators precedes systemic lupus erythematosus classification and improves prognostic accuracy of autoantibodies. J Autoimmun 2016;74:18293



Genetic Risk Factors

Environmental Triggers

- Hormones
- Microbes
- Ultraviolet light
- Diet
- Toxins
- Stochastic effects

Benign Autoimmunity

Autoantibody and Cytokine Production

Pathogenic Autoimmunity

Increased Autoantibody Production

BLyS, IL-2,IL-5, and G-CSF

Clinical Features

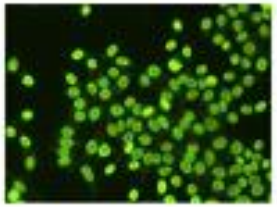
Cytokine Dysregulation and Activation of B and T Cells

pathogenic dysregulated autoimmune responses

- Type I IFN
- TNF
- IL-6
- Epigenetic changes
- Tissue damage

Self-sustaining feed-forward loop

SLE Classification



How to prevent latent SLE

Study suggested that early intervention with HCQ and/or prednisone may delay disease onset and slow accrual of autoantibody specificities

- In this study, individuals treated with HCQ before diagnosis had a longer time between the onset of the first clinical symptom and SLE classification than matched, HCQ-untreated SLE subjects (median times of 1.08 vs 0.29 years, respectively; P 5 .018).
- Additionally, individuals receiving both HCQ and prednisone (n 5 13) had a significantly longer time between initial clinical symptoms and SLE classification than individuals treated with only prednisone (n 5 14) before diagnosis (P 5 .03).
- Importantly, subjects treated with HCQ had a lower rate of autoantibody accumulation and decreased numbers of autoantibody specificities at and after SLE diagnosis.
- These findings indicate that preventative treatment in individuals at increased risk of SLE development may be beneficial and a preclinical study of HCQ in high-risk individuals for subsequent disease development is warranted

James JA, Kim-Howard XR, Bruner BF, et al. Hydroxychloroquine sulfate treatment is associated with later onset of systemic lupus erythematosus. Lupus 2007;16(6):401–9.

HCQ

- Presence of a low ANA titre on an isolated occasion might not require treatment
- Hydroxychloroquine treatment, this therapy might be used for individuals who have a composite serology (such as positivity for anti-dsDNA or specific anti- extractable nuclear antigen (ENA) antibodies) and/or low complement levels and high risk cytokine profiles because the risk of progression is higher in these patients than in patients with ANA positivity alone

Several drugs that reverse the pattern of epigenetic modifications have already been used to treat SLE

- Histone deacetylase inhibitors and DNA-demethylating drugs could also be a promising source of epigenetic therapies to treat SLE.
- Histone deacetylase inhibitors (HDAC) such as TSA have proved to be useful for relieving SLE disease in mice
- The effects of TSA on human T cells are immunosuppressive and reminiscent of the signaling aberrations that have been described in patients with SLE.
- Current evidence indicates that most of the genes that exhibit aberrant patterns of DNA methylation are hypomethylated, although gene-gene-specific hypermethylation cannot be ruled out. Therefore, a detailed analysis of DNA methylation at the gene level will serve to evaluate how useful DNA demethylating drugs could be.

Some of the mediators that were down-regulatedare involved in regulation of metabolism (resistin and leptin)

- Recent studies have shown that medications approved for the treatment of insulin-resistant diabetes may be beneficial in lupus , suggesting that dysregulation of metabolic pathways is involved in lupus pathogenesis.

Potential avenues for early intervention

- peroxisome proliferator-activated receptor γ (PPAR-γ) agonists, which are used clinically to enhance insulin sensitivity, have been found to reduce disease progression in mouse models of SLE when treatment is started before disease onset
- In lupus-prone mice, the PPAR-γ agonist rosiglitazone(Avandia) reduces lymphadenopathy, serum ANA titer, anti-dsDNA antibody levels, and renal disease when treatment is started before the onset of disease

Potential avenues for early intervention

- Lupus prone mice given metformin with the glucose metabolism inhibitor 2-deoxy-d-glucose during the early stages of disease showed significant reductions in splenomegaly, production of anti-dsDNA IgG and ANA, and immune complex deposition in the kidneys.

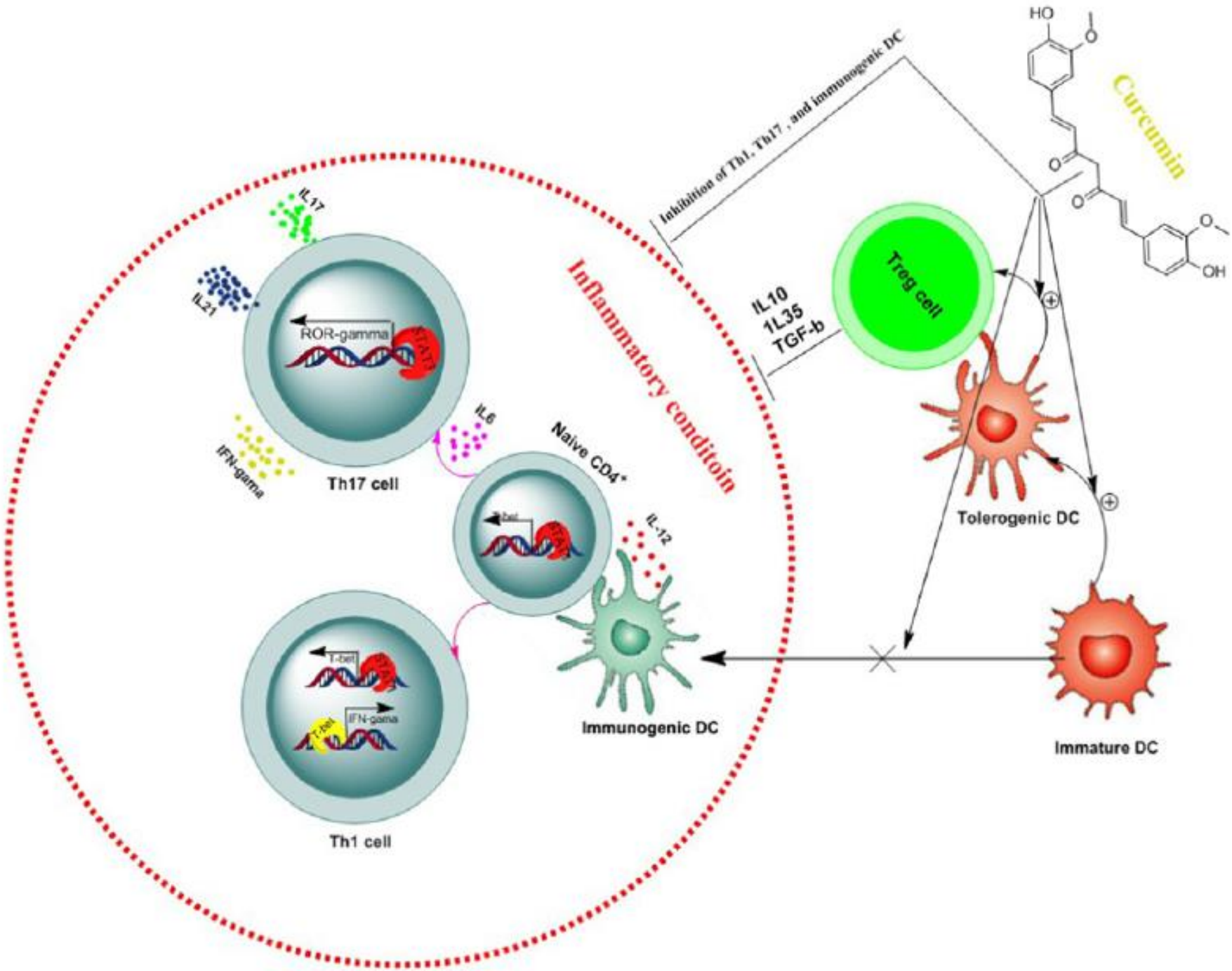
Green Tea Products

- Epidemiological evidence indicates that in comparison to the United States and Britain, the incidence of lupus is considerably lower in China and Japan, the two leading green-tea consuming countries. It is hypothesized that green tea polyphenols (GTPs) may be at least partly responsible for this geographical difference in lupus severity and prevalence. To support this theory, a number of molecular, cellular and animal studies have indicated that GTPs can provide protective effects against autoimmune reactions in salivary glands (particularly important in Sjögren's Syndrome) and in the skin by suppressing autoantigen expression and down-regulating inflammatory cytokines. These studies have not been replicated in humans but preliminary data appears promising. Further research would be needed to check out any beneficial effects of GTPs.

Curcumin

- Due to its well-established anti-inflammatory, antioxidant, antibacterial, hypoglycemic and wound healing effects
- Curcumin has been found to inhibit the maturation and function of DCs by reducing the expression of MHC-II and costimulatory molecules, prevents DC and T-cell contact, inhibition of IFN-γ production due to curcumin's effect of suppressing NF-κB activation and reduce Th17 cell responses

A.A. Momtazi-Borojeni et al. Curcumin: A natural modulator of immune cells in systemiclupus erythematosus/ Autoimmunity Reviews 17 (2018) 125–135


Inhibition of Th1, Th17 , and immunogenic DC
Curcumin
HO
O
O
O
OH
Inflammatory conditoin
IL17
IL21
ROR-gamma
STAT3
Th17 cell
IL6
IFN-gama
Naive CD4+
IL-12
T-bet
STAT4
IFN-gama
Th1 cell
Immunogenic DC
IL10
1L35
TGF-b
Treg cell
Tolerogenic DC
Immature DC

Curcumin

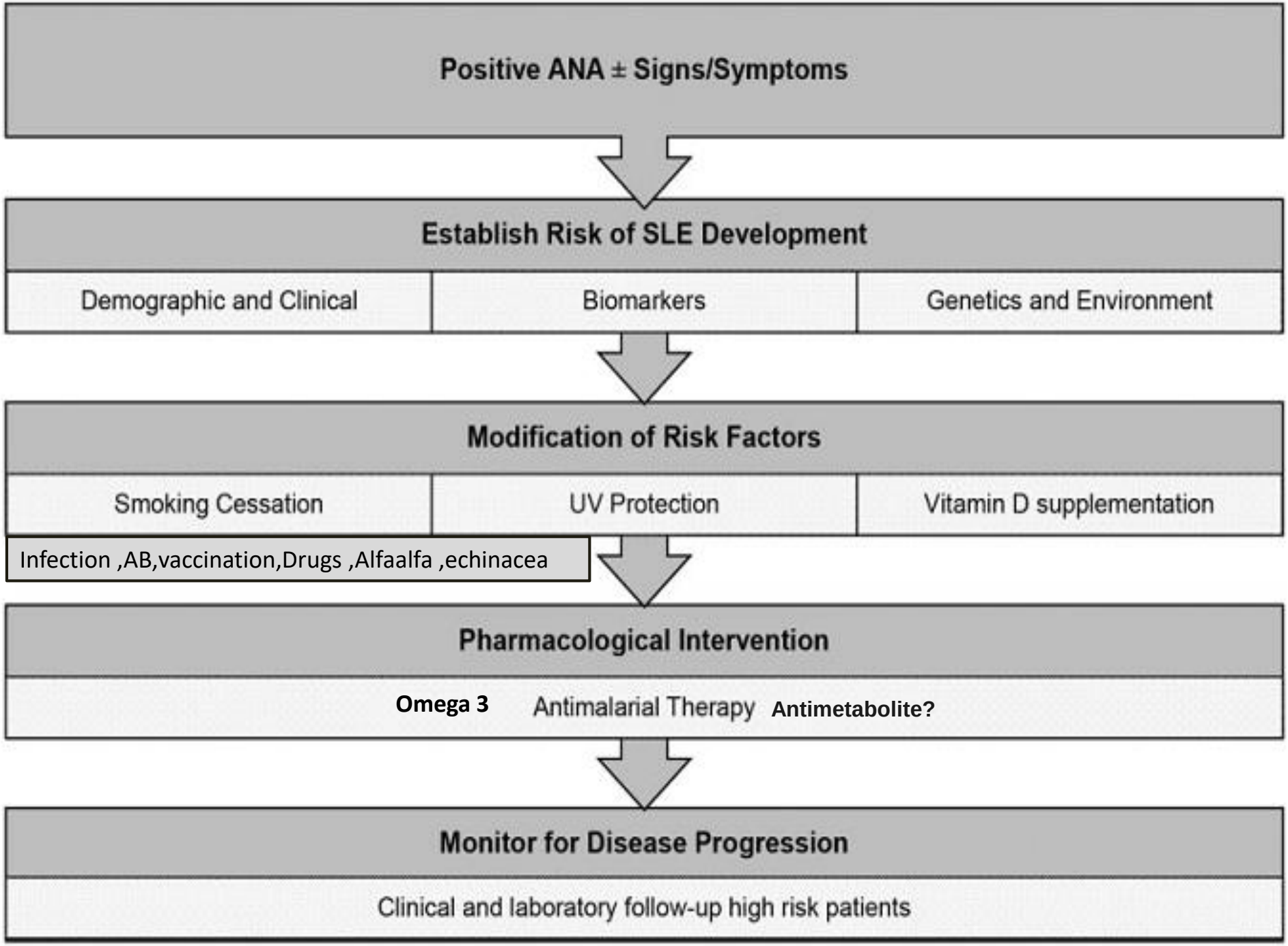
- In lupus nephritis is 500 mg daily for 3 months, which leads to a reduction in proteinuria, hematuria and blood pressure in SLE patients who have relapsing of intractable lupus nephritis
- Curcumin modulates pro-inflammatory cytokines, adhesion molecules and CRP, thus eliciting a beneficial anti-inflammatory effect in arthritis, by reducing pain and CRP level, and increasing the walking distance, at a dosage of 200 mg daily for 3 months
- curcumin supplementation is considered safe in up to 12 g daily
- In preclinical lupus?

A.A. Momtazi-Borojeni et al. Curcumin: A natural modulator of immune cells in systemiclupus erythematosus/ Autoimmunity Reviews 17 (2018) 125–135

omega-3 fatty acid

- Acts by reducing the formation of eicosanoids with inflammatory characteristics, since it competes with omega-6 fatty acids for the same enzymatic pathway, leading to the inhibition of TNF-, IL-1 and IL-6 synthesis and reducing the intercellular adhesion molecule-1(ICAM-1) expression.
- Overall, 5 of the 7 SLE studies indicated that fish oil supplementation modified and improved disease activity
- May be it useful in early stage of LUPUS

Umair Akbar,Omega-3 Fatty Acids in Rheumatic Diseases A Critical Review Journal of Clinical Rheumatology • Volume 23, Number 6, September 2017


Positive ANA ± Signs/Symptoms
Establish Risk of SLE Development
Demographic and Clinical
Biomarkers
Genetics and Environment
Modification of Risk Factors
Smoking Cessation
UV Protection
Vitamin D supplementation
Infection ,AB,vaccination,Drugs ,Alfaalfa ,echinacea
Pharmacological Intervention
Omega 3
Antimalarial Therapy
Antimetabolite?
Monitor for Disease Progression
Clinical and laboratory follow-up high risk patients


Thank You